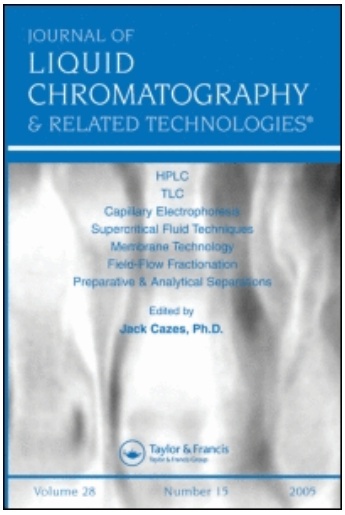

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THE SELECTIVITY BETWEEN GLYCINE AND TAURINE CONJUGATED BILE ACIDS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The dependence of parameter S on log k'_w in the retention equation log $k' = \log k'_w - S\varphi$ for glycine and taurine conjugated bile acids has been found for the first time to be two parallel lines in reversed-phase high-performance liquid chromatography (RP-HPLC). The difference in parameter log k'_w or S between glycine and taurine conjugates has been observed to be a constant, which therefore results in the constant selectivity factor between five different glycine and taurine conjugated bile acids in RP-HPLC.

INTRODUCTION

In recent years ,considerable attention has been directed towards the biodynamics of bile acids in patients with hepatobiliary diseases, serum bile acid concentration has been proved to be a sensitive indicator of liver dysfunction in a variety of diseases [1－2].

Bile acids usually occur naturally in conjugation with glycine or taurine. Reversed－phase high－performance liquid chromatography (RP－HPLC) has been widely used methods for identification and determination of individual bile acids and their conjugates in biological fluids [1－7], but up to now ,to the best of our knowledge, there is no report of any systematic study into the selectivity between the two types of the conjugates. In this work, we have described the selectivity between glycine and taurine conjugated bile acids based on the retention equation $\log k' = \log k'_w - S\varphi$ in RP－HPLC. We have found that the correlation of parameter S with $\log k'_w$ in the retention equation for glycine and taurine conjugated bile acids results in two parallel lines in RP－HPLC. The difference in parameter $\log k'_w$ or S between the glycine and taurine conjugates has been found for the first time to be a constant which therefore results in the constant selectivity between glycine and taurine conjugates at the fixed concentration of mobile phase despite a considerable variation in retention time for each pair of the compounds.

EXPERIMENTAL

1) APPARATUS

Chromatography was carried out with a Shimadzu LC－9A pump (Shimadzu Corporation, Kyoto, Japan) set at the flow rate of 1.0 ml/min, samples were loaded with a Rheodyne 7010 loop injection valve (Cotati, California, USA). The stainless－steel column (250 * 4.6mm i.d.) that con-

tained a Spherisorb C_{18} reversed phase packing material with 5 μm particle diameter was packed by National Chromatographic R. & A. Center (Dalian, China). Peaks were detected using a UVIDEC-100-I detector at 210 nm (Japan Spectroscopic Co. LTD, Tokyo, Japan). The eluent pH was measured by using Cole-Parmer Chemcadet 5986-50 pH meter (Taiwan, China). All HPLC measurement was carried out at room temperature.

2)REAGENTS

The conjugated bile acid standards were taurocholic acid (TC), taurochenodeoxycholic acid (TCDC), taurodeoxycholic acid (TDC), tauroursodeoxycholic acid (TUDC), taurolithocholic acid (TLC), glycocholic acid (GC), glycochenodeoxycholic acid (GCDC), glycodeoxycholic acid (GDC), glycoursodeoxycholic acid (GUDC), glycolithocholic acid (GLC) which were obtained from Colibiochem-Behring Company. All chemicals were of analytical grade.

3)CHROMATOGRAPHY

Separation of the bile acids was achieved using different composition of methanol in phosphate buffer. Mobile phase was prepared volumetrically from individually measured volumes of methanol and phosphate buffer. The eluent pH has been adjusted to pH of 5.50. Capacity factors were determined using equation $k' = (t_r - t_0)/t_0$, where t_r is the retention time of the bile acid, t_0 is the dead time of the column, which was measured as the retention time of the methanol peak.

RESULTS AND DISCUSSION

It has been generally accepted that the effect of the organic *modifier* concentration (φ) on the logarithm of the capacity factor (log k') in RP-

HPLC can be simply described by the retention equation (1) [8—10] as it is shown in the following:

$$log\ k' = log\ k'_w - S\varphi \qquad (1)$$

where parameter log k'_w is the capacity factor obtained by extrapolation of retention data from binary eluent to pure water. It mainly describes the difference in the interaction of solute—water and solute—stationary phase [9—11]. Parameter S is mainly determined by the molecular interaction between the solute and the mobile phase. It shows the difference in interaction between solute—water and solute—organic modifier and it is a constant for a particular solute even when column systems with different C_{18} packings are used [11]. Parameters log k'_w and S are well correlated with the solvatochromic parameters and thus are the function of the molecular structure [12].

Table 1 shows the capacity factors of ten conjugates at different methanol concentrations in RP—HPLC. The results of the linear regression analysis between log k' and φ are given in Table 2 where R is the regression coefficient. The linear regression of the experimental data in all cases is larger than 0.99 which strongly supports the validity of eqn. 1.

It has been found theoretically and experimentally that parameter S has the following linear relationship with log k'_w for a series of compounds with closely related structures [10—12]:

$$S = L_1 * log\ k'_w + L_2 \qquad (2)$$

where L_1 is a constant characterized the column system and mobile phase utilized. L_2 is a constant characterized the properties of the structural related compounds. Fig. 1 shows the general chemical structure of glycine and taurine conjugates.

It can be seen from Figure 1, the conjugated bile acids have separated into

Table 1

The capacity factors of ten major conjugated bile acids and the selectivity factors between glycine and taurine conjugates at different methanol concentrations in the eluent *

For the chromatographic conditions, see Experimental.

Bile acid	Proportion of methanol (v/v)(%)							
	80		75		70		65	
	k'	α	k'	α	k'	α	k'	α
GUDC	0.67	1.60	1.30	1.58	2.47	1.47	4.44	1.31
TUDC	0.42		0.82		1.68		3.29	
GC	1.13	1.66	2.24	1.58	4.28	1.49	7.79	1.27
TC	0.68		1.42		2.87		5.76	
GCDC	2.04	1.56	4.43	1.56	9.04	1.47	17.02	1.32
TCDC	1.31		2.84		6.15		12.88	
GDC	2.40	1.59	5.00	1.56	10.80	1.50	20.76	1.33
TDC	1.51		3.21		7.19		15.59	
GLC	4.30	1.56	9.65	1.57	21.71	1.49	45.71	1.35
TLC	2.76		6.14		14.53		33.75	

* Spherisorb—ODS, eluent: methanol—phosphate buffer, pH 5.5.

Table 2

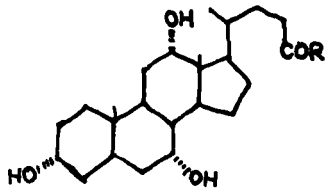
The parameters log k'_w, S in eq(1) given by linear regression of the experimental data in Table 1 and the values of $\triangle$log k'_w and $\triangle$S between glycine and taurine conjugates

For chromatographic conditions, see Experimental.

Bile acid	log k'_w	S	R[a]	$\triangle$log k'_w	$\triangle$S
GUDC	4.22	5.48	1.00	−0.19	−0.51
TUDC	4.41	5.99	1.00		
GC	4.54	5.59	1.00	−0.24	−0.59
TC	4.78	6.18	1.00		
GCDC	5.24	6.15	0.999	−0.18	−0.48
TCDC	5.42	6.63	1.00		
GDC	5.42	6.29	0.999	−0.18	−0.49
TDC	5.60	6.78	1.00		
GLC	6.13	6.86	1.00	−0.12	−0.41
TLC	6.25	7.27	1.00		

[a] R is the regression coefficient.

two types of structural related compounds, one is glycine conjugates, another is taurine conjugates, which was clearly illustrated in Figure 2. The dependence of parameter S on log k'_w has resulted in two parallel lines for glycine and taurine conjugated bile acids in RP−HPLC, which has agreed well with our theoretical assumption. Therefore S− log k'_w linear correlation analysis can be used to identify the structural similarity of the conjugated bile acids.



R=$NHCH_2COOH$ for glycine conjugates

R=$NH(CH_2)_2SO_3H$ for taurine conjugates

Fig. 1. General structure of the conjugated bile acids.

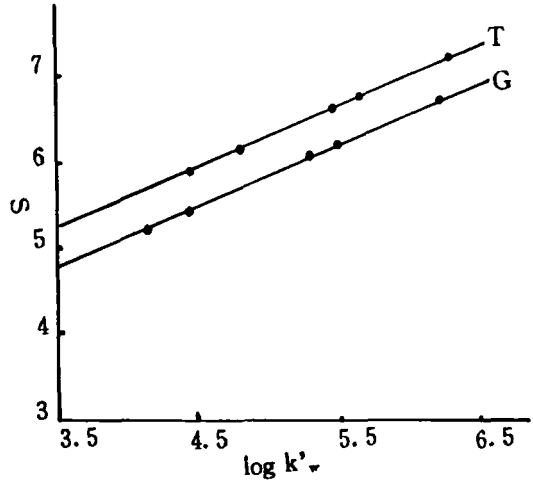


Fig. 2. The dependence of S on log k'_w in retention equation log $k' = \log k'_w - S\varphi$ for glycine (G) and taurine (T) conjugated bile acids in RP−HPLC. Parameters S and log k'_w were from Table 2. For chromatographic conditions, see Experimental.

On the other hand, by examinging log k'_w — values and S — values for glycine and taurine — conjugates in Table 2, we have found the rule that parameter S increases with log k'_w which thus results in an increase in retention[10]can only be observed for the structural related compounds.

The conjugation selectivity between glycine and taurine conjugates in RP—HPLC is defined as the ratio of capacity factor of glycine conjugated bile acid to that of taurine conjugated bile acid, as is shown by equation (3).

$$\alpha = k'(G)/k'(T) \qquad (3)$$

By combining of eqns(1) and (3), we have:

$$log\ \alpha = \triangle log\ k'_w - \triangle S \cdot \varphi \qquad (4)$$

As the difference in parameter log k'_w ($\triangle$log k'_w) or S ($\triangle$S) between glycine and taurine bile acids is a constant (see Table 2). Therefore there is the constant selectivity factor between five different glycine and taurine conjugates at the fixed eluent composition despite a considerable variation in retention time for each pair of compounds [7]. The contribution of the functional groups in the bile acid nucleus to the retention is the same whether the bile acid is conjugated with glycine or taurine. This conclusion may be generally true if there is no group interactions. The constant difference in log k'_w or S between glycine and taurine conjugates also implies that the parameters log k'_w and S are the function of molecular structure [12] and that the linear relationships between log k' and φ for each pair of the conjugates cross cach other at the same point (see Figure 3). This intersection point rule for the conjugated bile acids shows another feature of glycine and taurine conjugates.

The selectivity factors between glycine and taurine bile acids determined with various proportions of methanol in mobile phase are also listed in Table 1. As is seen from Table 1, the selectivity factors between glycine and taurine bile acids increase with increasing water content, but the selectivity fac-

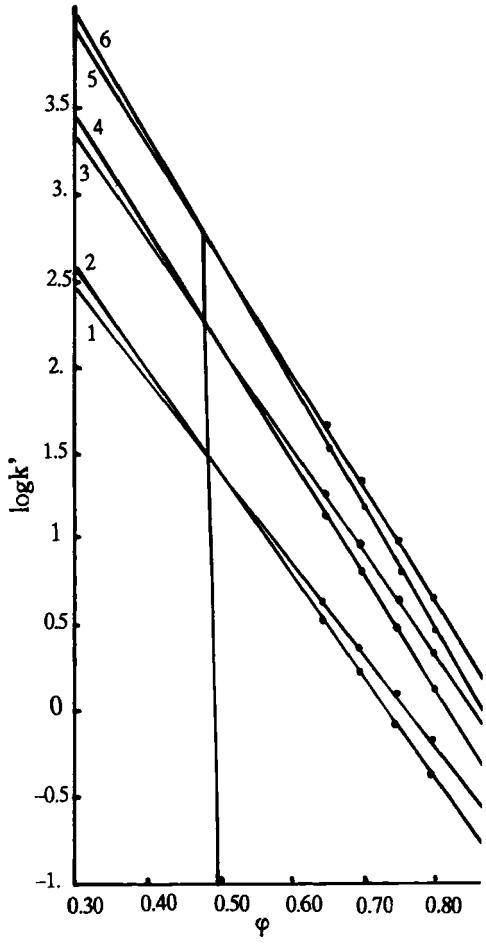


Fig. 3. Variation of the capacity factors with mobile phase composition for the conjugated bile acids on Spherisorb—ODS, methanol—0.01 M phosphate buffer, pH=5.5, peaks: 1=GUDC, 2=TUDC, 3=GCDC, 4=TCDC, 5=GLC, 6=TLC.

Table 3

The capacity factors of ten major conjugated bile acids and the selectivity factors between glycine and taurine conjugates determined with methanol—buffer as mobile phase using different stationary phases.
The experimental data are from ref. 4.

	System 1		system 2		system 3		system 4		system 5	
Bile acid	k'	α	k'	α	k'	α	k'	α	k'	α
GUDC *	3.15	1.30	2.02	1.62	3.06	1.33	2.00	1.43	2.20	1.55
TUDC	2.42		1.25		2.31		1.40		1.42	
GC	5.00	1.30	3.21	1.59	5.00	1.35	3.93	1.44	2.94	1.50
TC	3.84		2.02		3.71		2.72		1.96	
GCDC	10.20	1.30	6.13	1.63	10.35	1.36	8.11	1.45	6.23	1.57
TCDC	7.86		3.76		7.63		5.58		3.97	
GDC	11.78	1.32	7.07	1.66	11.94	1.37	9.68	1.47	7.13	1.59
TDC	8.96		4.25		8.69		6.57		4.49	
GLC	23.18	1.31	13.04	1.67	23.50	1.31	18.67	1.47	15.50	1.63
TLC	17.68		7.82		17.92		12.69		9.69	

* For abbreviations see Experimental.

System 1: μ—Bondapak C_{18}, eluent: methanol—0.02 M phosphate buffer, pH 4.2 (60:30).

System 2: μ—Bondapak C_{18}, eluent: methanol—acetonitrile—0.03 M phosphate buffer, pH 3.4 (60:10:30).

System 3: μ—Bondapak C_{18}, eluent: methanol—0.02 M sodium acetate (60:30) adjusted to pH 4.2 with phosphoric acid (solvent system A)

System 4: Supelcosil LC—18—DB, eluent: solvent system A.

System 5: Lichrospher CH—8, eluent: solvent system A.

tors between five glycine and taurine conjugates are identical at the fixed proportion of methanol. The conjugation selectivities at 80%, 75%, 70% and 65% methanol vary by 1.59±0.04, 1.57±0.01, 1.48±0.01 and 1.30±0.04 respectively (mean±S.D.).

Table 3 summarizes retention values for ten major conjugated bile acids and selectivity factors between glycine and taurine bile acids on different columns at a specific composition of mobile phase in RP－HPLC. As is seen in Table 3, despite a considerable variation in capacity factors for each pair of glycine and taurine conjugates, the selectivity factors between two types of the conjugates are independent of the type of bile acids. Retentions between glycine and taurine bile acids are controlled by the same dominant factor e. g. by glycine and taurine groups provided that there is no group interactions.

Peak identification is a relatively weak part of the analysis of the bile acids. The methods which have commonly used is based on the retention of the standards. The conjugation selectivity has been found to be used for peak identification of the conjugated bile acids in RP－HPLC without using any standards and has been proved to be a reliable method for peak identification of the conjugates[7]. Conjugation selectivity will find more and more uses in the peak identification of the conjugated bile acids in routine clinical analysis.

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